ORIGINAL ARTICLE

Serum Progesterone Measurement in Diagnosis of Ectopic Pregnancy

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Abstract

Aim: The implantation of the fertilized ovum outside the normal uterine cavity is termed an ectopic pregnancy (EP). Progesterone an important hormone in the regulation and maintenance of pregnancy has been studied with limited validation as a biomarker of this condition. The aim of this study was to determine whether a measurement of progesterone could discriminate an EP from a normal pregnancy.

Materials and methods: Serum levels of progesterone were measured by direct chemiluminescence in 140 women with EP and 140 women with normal pregnancy at Sri Ramachandra Medical College and Research Institute, Chennai, India. Statistical analyses were performed using SPSS software version 16.0, and a *p* value of less than 0.05 was considered significant.

Results: The mean progesterone level in EP was 6.4 ng/mL. This was significantly lower than the value of 24.6 ng/mL in normal pregnancy. Receiver operating characteristic (ROC) curve analysis revealed that at a cutoff of 16.22 ng/mL, progesterone was able to distinguish an EP from a normal pregnancy with a sensitivity of 98.6% and specificity of 87.1%.

Conclusion and clinical significance: Progesterone measurement in women in early pregnancy can be used to rule out an EP. Especially in the time window of 4–6 weeks of gestation where ultrasonography is inconclusive, we have to rely on biomarkers like progesterone to resolve the treatment modalities at the earliest.

Keywords: β-Human chorionic gonadotropin, Ectopic pregnancy, Gestational age, Pregnancy, Progesterone, Serial measurement, Serum biomarker, Ultrasonography.

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Introduction

Progesterone is the major hormone which regulates and maintains a normal pregnancy. It prepares the uterus for embedding the fertilized embryo, and its levels are maintained by the corpus luteum up to about 10–11 weeks of gestation and later taken over by the placenta. In an ectopic pregnancy (EP), the action of an abnormal corpus luteum early on leads to a decrease in the levels of progesterone. This was first documented in the late 1970s by Milwidsky et al.,[1] and the potential of progesterone to differentiate between an EP and a normal pregnancy was contemplated.

Starting then, measurement of serum progesterone had been proposed and studied as a noninvasive technique for diagnosis of an EP. Following the advent of a direct radioimmunoassay which yielded the results in less than 4 hours, its use in an emergency setup was advocated.[2,3] Numerous studies promoted the use of progesterone as a clinical adjunct in the diagnosis panel of EP.[4,5] However, it has not been brought into common usage as a biomarker for EP diagnosis along with serial β-human chorionic gonadotropin (β-hCG) measurement and ultrasonography.

The objective of this study was to ascertain whether the measurement of progesterone could differentiate an EP from a viable intrauterine pregnancy in 4–10 weeks of gestational age.

Materials and Methods

This prospective case–control study was conducted from April 2015 to August 2017 in 280 women between the age group of 19 years and38 years at Sri Ramachandra Medical College Hospital and Research Institute, Chennai, Tamil Nadu. A total of 140 cases were included sequentially from patients who were willing to participate in the study, admitted with an EP between 4 weeks and 10 weeks to the obstetrics and gynecology (OBG) inpatient department. The admitted women were followed up to confirm the diagnosis of an EP either with an ultrasound or later on when a laparotomy was performed for surgical treatment. A total of 140 controls were sequentially included from pregnant women who agreed to participate in this study, attending their first routine hospital booking visit between 4 weeks and 10 weeks of gestation in the OBG outpatient department.

The Institutional Ethics Committee of Sri Ramachandra Medical College and Research Institute, Chennai, approved the study, and the study was performed in accordance with its recommendations and that of Helsinki Declaration of 1975 that was revised in 2000. All women participating in this study gave a written informed consent.

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History and clinical characteristics of all women were recorded including maternal age, parity, and gravidity. Gestational age was obtained from first trimester ultrasound reports, either transvaginal or transabdominal whichever was performed for diagnosis in the women. A venous blood sampling was carried out by a trained phlebotomist on all study subjects, collected in serum separator tubes, spun at 3,000 rpm for 15 minutes, and serum was separated. Progesterone was assayed by direct chemiluminescence on Beckman Coulter Unicel Dxl 800 immunoassay system.

Results

Statistical Package for the Social Sciences statistical software package for Windows, version 16.0 (SPSS, Inc., Chicago, IL, USA), was the statistical software used, and a two-tailed p value < 0.05 was considered significant.

The average maternal age in normal pregnant women was 24.9 ± 3.5 years, and in EP, it was 25.6 ± 3.3 years. The average gestational age in normal pregnant women was 6.6 ± 1.5 weeks, and in EP, it was 6.3 ± 1.6 weeks. For these baseline characteristics, Student's t test was used to compare between normal pregnancy and EP (Table 1), and a p value of 0.073 was obtained for maternal age and 0.131 for gestational age. There was no statistical difference between the cases and controls with regard to maternal age and gestational age. The method of confirmation of diagnosis in normal pregnancy and EP is tabulated in Table 2.

The mean concentration of progesterone was found to be 24.6 ± 8.8 ng/mL in normal pregnancy, and in EP group, it was 6.4 ± 3.8 ng/mL. Independent Student's t test was used to assess the disparity between normal pregnant women and EP group and was found to be statistically significant at $p = 0.000$ (Table 3).

The women were divided into two groups based on their gestational age: the first group comprised women in 4–6 weeks of gestation and the second group comprised women in 7–10 weeks gestation. Independent Student's t test was used to assess the difference between normal pregnant women and EP women in both groups. The difference was found to be statistically significant with a p value of 0.000 (Tables 4 and 5).

The receiver operating characteristic (ROC) curve analysis of progesterone demonstrated area under the curve of 0.990 to discriminate EP from viable intrauterine pregnancy (Fig. 1). The sensitivity and specificity of this test at a cutoff value < 16.22 ng/mL was 98.6 and 87.1%, respectively. The positive predictive value is 88.5 and the negative predictive value is 98.4.

Table 1: Demographic features of study participants

	Normal pregnancy (n = 140)	*EP (n = 140)*	*p value*
Maternal age (years)	24.9 ± 3.5	25.6 ± 3.3	0.073
Gestational age (weeks)	6.6 ± 1.5	6.3 ± 1.6	0.131

Table 2: Method of confirmation of diagnosis

	Ultrasonography	*Laparotomy*
Normal pregnancy	140	Not applicable
EP	113	27

Table 3: Comparison of progesterone between normal pregnancy and ectopic pregnancy

Unit of measurement: ng/mL	*Normal pregnancy*	*EP*
Progesterone	24.6 ± 8.8	6.4 ± 3.8
Minimum	10.32	0.58
Maximum	53.54	20.07

p value = 0.000

Discussion

The diagnosis of a woman presenting to the emergency with amenorrhea, pain abdomen, and bleeding per vaginum is diverse in the first trimester ranging from miscarriages, blighted ovum, molar pregnancy, and missed abortion to EP. Among these, higher incidence in EP in recent times is seen owing to assisted reproductive technologies, pelvic inflammatory disease, and many others.[6,7] Numerous biomarkers have been studied in aiding in the diagnosis of EP though with limited validation. Among these, progesterone is exceptionally imperative in readying the uterus for implantation and preservation of pregnancy. Progesterone with the chemical formula $C_{21}H_{30}O_2$ and a molecular weight of 314.469 g/mol has a biological half-life of 34.8–55.13 hours.[8] In this study, progesterone has been studied as a diagnostic marker for EP.

The subjects in both the normal pregnancy group and EP group were matched with regard to maternal age and gestational age (Table 1). The average gestational age of normal pregnant women

Table 4: Serum progesterone levels in normal pregnancy and ectopic pregnancy in the gestational age of 4–6 weeks

Unit of measurement: ng/mL progesterone	*Normal pregnancy (n = 87)*	*EP (n = 85)*
Mean	20.48	4.62
SD	3.79	2.26
Range	10.32–29.53	0.58–13.59

p value 0.000; SD, standard deviation

Table 5: Serum progesterone levels in normal pregnancy and ectopic pregnancy in the gestational age of 7–10 weeks

Unit of measurement: ng/mL progesterone	*Normal pregnancy (n = 53)*	*EP (n = 55)*
Mean	32.74	9.08
SD	8.63	4.22
Range	21.57–53.54	0.99–20.07

p value = 0.000; SD, standard deviation

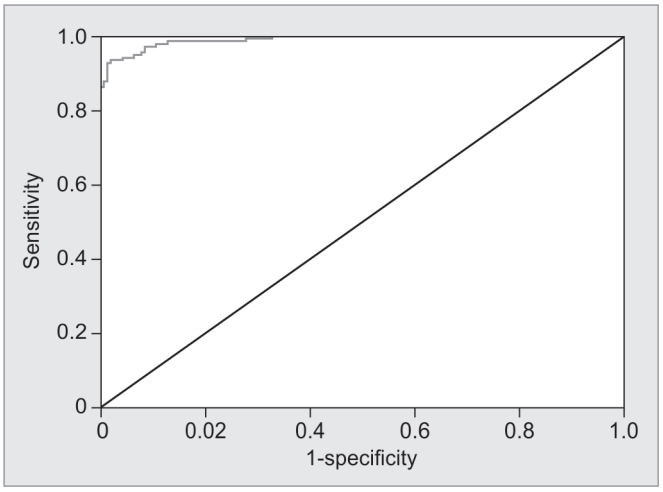

Fig. 1: Receiver operating characteristic curve analysis for progesterone in discriminating ectopic pregnancy from normal pregnancy

was 6.6 weeks and of EP reported in this study was 6.3 weeks. The mean progesterone level in EP was 6.4 ng/mL. This was significantly lower than the value of 24.6 ng/mL observed in normal pregnancy (Table 2). Similarly, Graziosi et al. showed that the levels in EP were lower compared with viable intrauterine pregnancy.[9]

A transvaginal ultrasound can detect a gestational sac as early as 5 weeks; the fetal pole is visualized at 6 weeks, and the fetal heart beat is picked up by around 7 weeks of gestation.[10] The initial 4–6 weeks of conception are, therefore, critically important from a diagnostic viewpoint, to determine the presence of an extrauterine implantation of blastocyst. So, the subjects in this study were divided into two groups based on gestational age: one from 4 weeks to 6 weeks and the other from 7 weeks to 10 weeks (Tables 4 and 5). A significant decrease in the average progesterone levels between normal pregnancy and EP women in both the groups was also observed.

The ROC curve analysis (Fig. 1) revealed that at a cutoff 16.22 ng/mL, progesterone was able to distinguish an EP from a viable intrauterine pregnancy with a sensitivity and specificity of 98.6 and 87.1%, respectively. Carson and Buster established a threshold value of 20 ng/mL to exclude failed pregnancies.[11] Yeko et al. predicted a level of 15 ng/mL, above which they say there is 100% impossibility of an EP.[12]

In the meta-analysis conducted in 2012 by Verhaegen et al., at values from 3.2 to 6 ng/mL, progesterone was able to tell apart a nonviable pregnancy with a sensitivity and specificity of 74.6 and 98.4%, respectively, despite it not being able to discern EP from other anomalous intrauterine pregnancies.[13] However, the cutoff established in this study seems to be higher than that reported by Verhaegen et al. and closer to that reported by Carson and Buster and Yeko et al. The meta-analysis conducted by Mol et al. also quote that it is safe to exclude normal pregnancy at a cutoff of 5 ng/mL,[14] and Al-Bayati et al. quote a cutoff of 11.7 ng/mL.[15] A disparity in the population of study could have been the reason for marked difference in the cutoff values quoted. Besides, a limitation in this study was that other abnormal intrauterine pregnancies have not been taken into consideration.

By decreasing the threshold value to 10.17 ng/mL of progesterone in this study, 100% specificity can be attained, but the sensitivity drops to 86.4%. This cutoff can be used when progesterone is used as a diagnostic test. And by increasing the threshold to 20.08 ng/mL of progesterone, 100% sensitivity can be obtained, but the specificity drops to only 67.1%. This can be used when progesterone is used in screening when EP is suspected. So, for subjects whose progesterone levels are in between these two cutoffs, a careful observation is necessary to reach the diagnosis.

National Institute for Clinical Excellence clinical guideline no. 126 on "Ectopic Pregnancy and miscarriage: diagnosis and initial management" advocates taking two serum β-hCG measurements as near as possible to 48 hours apart but no earlier in women with a pregnancy of unknown location.[16] In women with change in serum β-hCG concentration between 50% decline and 63% rise, careful monitoring is advocated. The guidelines also state that progesterone cannot be used as an adjunct to serial β-hCG measurement in women with a pregnancy of unknown location. However, the time window of 48 hours required for serial β-hCG measurement may pose a risk of rupture and hemorrhage impairing future fertility of the woman. In cases where both ultrasound and β-hCG measurements may not be able to conclude on the diagnosis, usage of progesterone measurement may go a long way in reducing the time and financial burden on this critical condition. Most previous studies do not have a record of the gestational age of the women participating in their study. In this study, the gestational age was recorded, and a variation in the values of progesterone with an increase in gestational age was noted in normal pregnancy and EP. Increasing the number of women in each gestational age group can help in establishing reference values for normal pregnancy and cutoff values for EP. This may further increase the usage of this marker in a clinical setup.

Conclusion and Clinical Significance

Ectopic pregnancy, considered a life-threatening condition, has seen an increase in incidence despite a decline in its mortality rate. Particularly in the time window of 4–6 weeks of gestation where ultrasonography is inconclusive and serial β-hCG measurement at an interval of 48 hours poses a risk of complications, adding progesterone measurement could help in reaching the diagnosis at the earliest.

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